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# Redox Potentials of Oligopeptide/$Fe_4S_4^{2+}$ Complexes. Remarkable Positive Shift of the Redox Potential with (Benzyloxycarbonyl)-L-Cys-Gly-L-Ala-L-Cys-OMe as Chelating Ligands

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$[Fe_4S_4(Z\text{-Cys-Gly-Ala-Cys-OMe})_2]^{2-}$ (Z = benzyloxycarbonyl) (**1**) and $[Fe_4S_4(Z\text{-Cys-Ile-Ala-Cys-OMe})_2]^{2-}$ (**2**) were synthesized from $[Fe_4S_4(S\text{-}t\text{-Bu})_4]^{2-}$ as [4Fe-4S] ferredoxin model complexes having an invariant sequence of Cys-X-Y-Cys (X, Y = amino acid residues) of bacterial ferredoxins. The chelation of Cys-X-Y-Cys to $Fe_4S_4^{2+}$ was established by $^1H$ NMR and CD spectra. A remarkable positive shift of redox potential of **1** in dichloromethane was found at lower temperatures (−0.93 V vs. SCE at 24 °C and −0.80 V vs. SCE at −42 °C), while **2** exhibited no such temperature dependence (−1.00 V vs. SCE at 30 °C and −0.96 V vs. SCE at −33 °C). **1** and **2** in DMF exhibit redox potentials at −0.95 and −1.01 V (vs. SCE), respectively, at −40 and 30 °C. The presence of a Gly residue adjacent to the Cys thiolato group was found to be advantageous for the formation of a preferable conformation for an NH---S hydrogen bond, which is probably one of the important factors for the more positive redox potential of native ferredoxins.

## Introduction

The redox potential of ferredoxin is one the crucial factors for smooth electron transfer in many biological systems.[1,2] The electrochemical properties of synthetic $[Fe_4S_4]^{n-}$ ($n$ = 2, 3) complexes with alkane or arenethiolato ligands have been studied by Holm's group.[2-4] The difference between native ferredoxin and the model complexes have been discussed in terms of redox potentials,[5] redox stabilities,[6] and electron-transfer rates.[7] These differences have been considered to be caused by unique chemical environments of peptide chains around the $Fe_4S_4^{2+}$ cluster[6] in native ferredoxin.

We have systematically studied the chemical role of invariant amino acid residues around $Fe_4S_4^{2+}$ core in the peptide sequences of bacterial ferredoxins. Previously we reported the importance of a Cys-Gly-Ala fragment of $[Fe_4S_4(Z\text{-Cys-Gly-Ala-OMe})_4]^{2-}$ in the formation of an NH---S hydrogen bond in nonpolar solvent.[8] The tetrapeptide fragments of Cys-X-Y-Cys (X, Y = amino acid residues) have been found in the metal binding sites of many metal thiolato proteins, e.g. *C. pasteurianum* rubredoxin,[9] *Spirulina platensis* ferredoxin,[10] *P. aerogenes* ferredoxin, horse liver alcohol dehydrogenase (E-chain),[11] *E. coli* aspartate carbamoyltransferase,[12] and renal metallothionein.[13] We have studied the synthesis and the characterization of various complexes of Pd(II),[14] Fe(II),[15] Fe(III),[16] Co(II),[17] or Mo(IV)[18] containing tetrapeptides (Z-Cys-X-Y-Cys-OMe) with relevance to the metal thiolate proteins.

This paper presents a study on the electrochemical properties of $Fe_4S_4^{2+}$ complexes of Cys-containing tetrapeptides, $[Fe_4S_4(Z\text{-Cys-X-Y-Cys-OMe})_2]^{2-}$. Spectroscopic characterization using $^1H$ NMR, CD, and visible spectra also revealed unique properties for the chelating tetrapeptide complexes. The model complexes having Cys-Gly-Gly-Cys ligands were already synthesized by Holm's[19] and Rydon's groups.[20] However, the Gly-Gly fragment should have no conformational restriction in the macroring chelation. No example of this sequence has been found in native metalloproteins. Recently, a [4Fe-4S] ferredoxin model complex of Cys-Gly-Cys was synthesized that seems to have extremely restricted chelating structure or to have a nonchelating oligomeric one, depending on the conditions.[21] The identity of the two amino acid residues (X and Y) interposed between the two Cys residues is thus important since the X-Y fragment is invariant in many metalloproteins, e.g. Cys-Gly-Ala-Cys in bacterial ferredoxins.

## Experimental Section

All operations were carried out under argon. All solvents were purified by distillation. In particular solvents, e.g. $Me_2SO$, dichloromethane, and DMF (*N*,*N*-dimethylformamide), for electrochemical measurements were purified by the reported procedures.[8] $[NEt_4]_2[Fe_4S_4(S\text{-}t\text{-Bu})_4]$ was prepared according to literature methods.[22]

**Preparation of Peptides. *N*-(Benzyloxycarbonyl)-*S*-(acetamidoethyl)-L-cysteinylglycine.** *N*-(Benzyloxycarbonyl)-*S*-(acetamidomethyl)-L-cysteinylglycine methyl ester (15 g, 0.083 mol), which was prepared from *N*-(benzyloxycarbonyl)-*S*-(acetamidomethyl)-L-cysteine and glycine methyl ester, was dissolved in methanol (150 mL). To the solution was added 25 mL (0.05 mol) of aqueous 2 N NaOH at 0 °C. After standing at room temperature for 4 h, the solution was neutralized by the addition of aqueous 2% HCl. The solution was concentrated under reduced pressure and gave a white solid with the addition of an excess of aqueous 2% HCl. The crude product was collected with filtration, washed with aqueous 2% HCl and ether, and dried in vacuo. The product was recrystalized from methanol–ether: yield 13.4 g (92%); mp 168–171 °C.

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**L-Alanyl-*S*-(acetamidomethyl)-L-cysteinyl Methyl Ester Hydrochloride.** (*tert*-Butyloxycarbonyl)-L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl methyl ester (1.24 g, 0.0033 mol) was dissolved in 20 mL of HCl-saturated ethyl acetate at 0 °C giving a white precipitate. After 2 h, 100 mL of ether was added to the solution, and the white precipitate was collected with decantation. The white solid obtained was dried over KOH in vacuo.

***N*-(Benzyloxycarbonyl)-*S*-(acetamidomethyl)-L-cysteinylglycyl-L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl Methyl Ester (Z-Cys(Acm)-Gly-Ala-Cys(Acm)-OMe).** A DMF solution of L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl methyl ester hydrochloride (0.013 mol) was mixed with a DMF solution of *N*-(benzyloxycarbonyl)-*S*-(acetamidomethyl)-L-cysteinylglycine (3.7 g, 0.013 mol) by vigorous stirring at −10 °C. To the solution was added 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide at 0 °C. The solution was neutralized with *N*-methylmorpholine and stirred overnight at room temperature. The solution was concentrated under reduced pressure. To the residue was added saturated aqueous NaCl. The products were extracted with ethyl acetate and washed successively with 4% $NaHCO_3$, water, 2% aqueous HCl, and water. After concentration under reduced pressure, the residue obtained was recrystallized from methanol–ether: yield 4.7 g (53%); mp 190–190 °C; $[\alpha]^{25}_D$ −27.5 (*c* 0.106, DMF). Anal. Calcd for $C_{26}N_{38}O_9N_6S_2$: C, 49.99; H, 6.13; N, 13.45. Found: C, 48.18; H, 5.98; N, 12.72.

**(*tert*-Butyloxycarbonyl)-L-isoleucyl-L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl Methyl Ester.** A solution of (*tert*-butyloxycarbonyl)-L-isoleucine (0.013 mol) in tetrahydrofuran (23 mL) was dried with $Na_2SO_4$ and cooled to −20 °C. To the solution were added triethylamine (1.85 mL, 0.013 mol) and isobutylchloroformate (1.73 mL, 0.013 mol) with vigorous stirring at −15 °C. After 15 min, a mixture of L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl methyl ester hydrochloride (0.013 mol) and triethylamine (1.85 mL, 0.013 mol) was added at −20 °C. The solution was allowed to stand at room temperature overnight after being stirred for 3 h at −15 °C. The solution was concentrated under reduced pressure, and water was added to the residue, giving a white solid, which was washed successively with 4% aqueous $NaHCO_3$, water, 10% aqueous citric acid, and water and dried over $P_2O_5$ in vacuo: yield 4.0 g (82%); mp 169–171 °C; $[\alpha]^{25}_D$ −12.1 (*c* 0.083, DMF). Anal. Calcd for $C_{21}H_{38}N_4O_7S_2$: C, 51.41; H, 7.81; N, 11.42. Found: C, 51.13; H, 7.77; N, 10.71.

***N*-(Benzyloxycarbonyl)-*S*-(acetamidomethyl)-L-cysteinyl-L-isoleucyl-L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl Methyl Ester.** (*tert*-Butyloxycarbonyl)-L-isoleucyl-L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl methyl ester (3.0 g, 0.0061 mol) was dissolved in HCl-saturated acetic acid (20 mL) with vigorous stirring. After standing at room temperature for 2 h, the solution was concentrated under reduced pressure. A white solid obtained was washed with ether and dried over KOH in vacuo. A solution of *N*-(benzyloxycarbonyl)-*S*-(acetamidomethyl)-L-cysteine (1.89 g, 0.0066 mol) in terahydrofuran (12 mL) was cooled at −20 °C. To the solution were added triethylamine (0.86 mL, 0.0066 mol) and isobutylchloroformate (0.80 mL, 0.0066 mol) at −15 °C. After 15 min, a solution of L-isoleucyl-L-alanyl-*S*-(acetamidomethyl)-L-cysteinyl methyl ester hydrochloride (0.0066 mol) and triethylamine (0.86 mL, 0.0066 mol) in DMF (4 ml) and chloroformate (12 mL) was added with vigorous stirring for 1 h at −20 °C. The crude product was obtained by the same method described for the synthesis of Z-Cys(Acm)-Gly-Ala-Cys-(Acm)-OMe. Recrystalization was carried out from DMF–ether: yield 3.7 g (86%); mp 210–212 °C; $[\alpha]^{25}_D$ −32.4 (*c* 0.0142, DMF). Anal. Calcd for $C_{30}H_{48}N_6O_9S_2$: C, 51.41; H, 6.90; N, 11.91. Found: C, 50.81; H, 6.88; N, 11.70.

**Mercury(II) Peptide Complexes.** To a $Me_2SO$ solution (2 mL) of the corresponding peptide (1.0 mmol) was added a solution of $HgCl_2$ (2.0 mmol) in $Me_2SO$. The solution was allowed to stand overnight at room temperature. About 10 mL of water was added to the solution, giving a white solid, which was collected by filtration, washed with water, and dried over $P_2O_5$ in vacuo. $Hg_2Cl_2$(Z-Cys-Gly-Ala-Cys-OMe) Raman data for Hg–S and Hg–Cl: 280, 319 $cm^{-1}$. $Hg_2Cl_2$(Z-cys-Ile-Ala-cys-OMe) Raman data for Hg–S and Hg–Cl: 298, 315 $cm^{-1}$. These two characteristic Raman bands indicate the presence of an S–Hg–Cl bond.

**Synthesis of SH-Deblocked Peptides.** A Hg(II) peptide complex (0.8 mmol) was dissolved or dispersed in methanol (200 mL). Hydrogen sulfide gas was bubbled through the solution for 15 min at room temperature, giving a black precipitate. The mixture was allowed to stand overnight with stirring. After filtration of the precipitate, the filtrate was concentrated under reduced pressure to give a white solid. The crude peptide was recrystallized from methanol–ether. The determination of SH group in these peptides was carried out by a spectroscopic titration with 2,2′-dithiobis(5-nitropyridine).[23]

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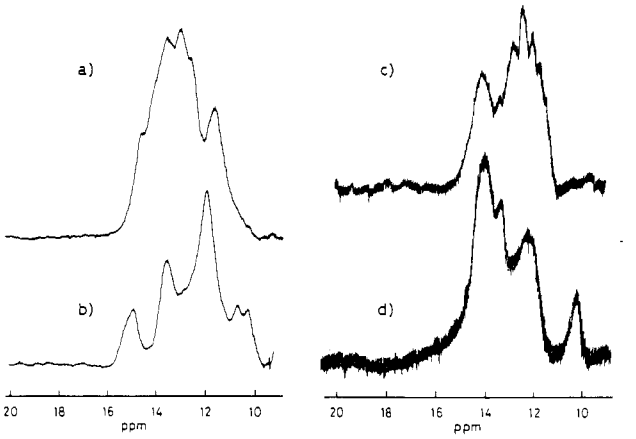


**Figure 1.** $^1H$ NMR spectra (400 MHz) of Cys $C_\beta H_2$ regions of (a) $[NEt_4]_2[Fe_4S_4(Z\text{-Cys-Gly-Ala-Cys-OMe})_2]$ in $Me_2SO$-$d_6$ and (b) in dichloromethane-$d_2$ and of (c) $[NEt_4]_2[Fe_4S_4(Z\text{-Cys-Ile-Ala-Cys-OMe})_2]$ in $Me_2SO$-$d_6$ and (d) in dichloromethane-$d_2$.

**Synthesis of $[NEt_4]_2[Fe_4S_4(Z\text{-Cys-X-Ala-Cys-OMe})_2]$ (X = Gly or Ile) Complexes.** To a solution of $[NEt_4]_2[Fe_4S_4(S\text{-}t\text{-Bu})_4]$ (50 mg, 0.056 mmol) in DMF was added a solution of Z-Cys-Gly-Ala-Cys-OMe (112 mg, 0.223 mmol) in DMF at room temperature. The solution was concentrated under reduced pressure to remove *tert*-butyl mercaptan and DMF. Addition of ether resulted in formation of a black solid. Purity of the [4Fe-4S] complexes was determined by the method reported by Gillum et al.[24] and by the ratio of $^1H$ NMR peak integrals of methylene protons in benzyloxycarbonyl and tetra-*n*-butylammonium protons. $[NEt_4]_2[Fe_4S_4(Z\text{-Cys-Ile-Ala-Cys-OMe})_2]$ was synthesized by the same method described above.

**Physical Measurements.** $^1H$ NMR spectra were recorded at 21 °C on a JEOL JNM-GX 400 spectrometer with tetramethylsilane as an internal standard. The sample concentrations were about 30 mg/mL. Absorption spectra were obtained on a Jasco UVDEC-5A instrument. CD spectra were measured on Jasco J-40 spectropolarimeter. The values of $\epsilon$ and $\Delta\epsilon$ are given in units of $M^{-1}$ $cm^{-1}$. Raman spectra were obtained so as to detect Hg–S and Hg–Cl bonds in Hg(*II*)/Cys-containing peptide complexes by using a Jasco R-800 spectrometer at a 488.0-nm excitation line. Electrochemical measurements were carried out by a Yanaco P8-CV instrument with a three-electrode system using a glassy-carbon working electrode, platinum-wire counter electrode, and a saturated calomel electrode separated by glass frits. Redox potentials ($E_p$) were estimated as the average of $E_{pa}$ and $E_{pc}$.

## Results

Synthesis of $[NEt_4]_2[Fe_4S_4(Z\text{-cys-X-Ala-cys-OMe})_2]$ (X = Gly, Ile) was carried out by using the ligand exchange method established by Holm's group.[22] This method has an advantage for the complete reaction as exemplified in the following scheme, which can be readily monitored by removal of *t*-BuSH in vacuo:

$$[Fe_4S_4(S\text{-}t\text{-Bu})_4]^{2-} + 2Z\text{-Cys-X-Ala-Cys-OMe} \rightarrow [Fe_4S_4(Z\text{-Cys-X-Ala-Cys-OMe})_2]^{2-} + 4t\text{-BuSH}$$

X = Gly, Ile

**$^1H$ NMR Spectra (400 MHz) of $[NEt_4]_2[Fe_4S_4(Z\text{-Cys-Gly-Ala-Cys-OMe})_2]$ (1) and $[NEt_4]_2[Fe_4S_4(Z\text{-Cys-Ile-Ala-Cys-OMe})_2]$ (2).** Figure 1 shows the $^1H$ NMR spectra (400 MHz) of the $C_\beta H_2$ region of $[Fe_4S_4(Z\text{-Cys-X-Ala-Cys-OMe})_2]^{2-}$ (X = Gly, Ile) and the related tripeptide complexes in $Me_2SO$-$d_6$ at 21 °C. The signals of $C_\beta H_2$ contact-shifted through H–C–S–Fe by the $Fe_4S_4^{2+}$ core are detectable in the region from 10 to 17 ppm.[25] Usually the $C_\beta H_2$ signals of Cys-containing peptides are observed at about 3.0 ppm, for example at 3.2 ppm for $[Hg_2Cl_2(Z\text{-Cys-X-Ala-Cys-OMe})]$. The $Fe_4S_4^{2+}$ complex of a tripeptide having one Cys

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**Table I.** Solvent Dependence of LMCT Absorption Maxima of $[Fe_4S_4(Cys\text{-}peptide)_n]^{2-}$ Complexes ($n$ = 2 or 4)

| complexes | LMCT absorption max, nm ($\epsilon$, $M^{-1}$ $cm^{-1}$) | |
|---|---|---|
| | DMF | $CH_2Cl_2$ |
| $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]$ | 409 (15 200) | 399 (15 900) |
| $[NEt_4]_2[Fe4S_4(Z\text{-}Cys\text{-}Ile\text{-}Ala\text{-}Cys\text{-}OMe)_2]$ | 414 (14 200) | 402 (12 100) |
| $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}OMe)_4]$ | 402 (16 100) | 402 (15 300) |
| $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}OMe)_4]$ | 406 (14 800) | 390 (16 000) |

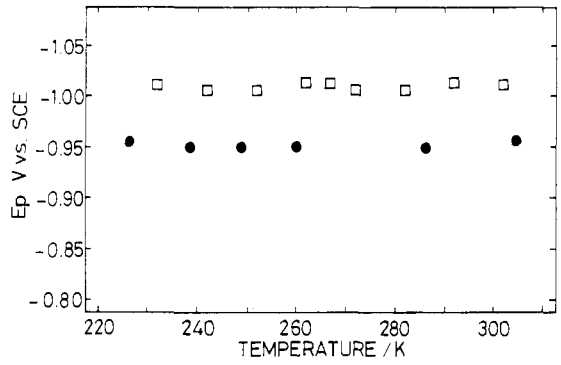


**Figure 2.** Temperature dependence of redox potentials of $[Fe_4S_4(Z\text{-}Cys\text{-}X\text{-}Ala\text{-}Cys\text{-}OMe)_2]^{2-}$ (X = Gly, Ile) in DMF: (●) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]$; (□); $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Ile\text{-}Ala\text{-}Cys\text{-}OMe)_2]$.

residue, $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Ile\text{-}Ala\text{-}OMe)_4]$, exhibited Cys $C_\beta\text{-}H_AH_B$ signals at 12.7 and 13.4 ppm as an AB part of an ABX spin system in $CD_2Cl_2$ or $Me_2SO\text{-}d_6$. The large difference ($\Delta\delta$ = 0.7 ppm) between the chemical shifts of $H_A$ and $H_B$ is caused by the different environment of the two protons induced by the adjacent asymmetric carbon atoms. Two $C_\beta H_2$ signals for $[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}OMe)_4]^{2-}$ and $[Fe_4S_4(Z\text{-}Ala\text{-}Cys\text{-}OMe)_4]^{2-}$ are observed each at 11.5 and 12.5 ppm in $Me_2SO\text{-}D_6$ and are assignable to geminal diastereotopic protons. The $Me_2SO\text{-}d_6$ solutions of **1** and **2** showed complex signals from 12.0 to 14.5 ppm. However, a $CD_2Cl_2$ solution of **2** exhibited mainly four separate signals at 10.5, 12.0, 13.5, and 15.0 ppm, although spectra for a $CD_2Cl_2$ solution of **1** did not show a broad multiplet in the same region as those in $Me_2SO\text{-}d_6$.

**Absorption Spectra of 1 and 2.** The ligand–metal charge-transfer (LMCT) absorptions of the peptide model complexes arund 400 nm are known to be sensitive to the dielectric constants of solvents.[21,26] A dipeptide complex, $[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}OMe)_4]^{2-}$, has a LMCT absorption maximum at 402 nm in either DMF or dichloromethane. However, the $Fe_4S_4^{2+}$ tetrapeptide complexes in dichloromethane showed a blue-shifted LMCT absorption maximum at 392–402 nm, as listed in Table I. The blue shift of LMCT absorption maxima around 390–420 nm has been pointed out to increase with an increase in dielectric constant of the solvent.[22,26] The tetrapeptide complexes exhibit a blue-shifted LMCT absorption maximum even in dichloromethane having a dielectric constant lower (8.93) than that of DMF (36.7). The unusual blue shift in dichloromethane is likely due to NH---S hydrogen bonding in $[Fe_4S_4(SR)_4]^{2-}$.

**Solvent and Temperature Dependence of the Redox Potentials in 1 and 2.** The effect of an X-Y segment interposed in the tetrapeptide ligands was particularly evident in the redox potentials of 3–/2–states. Figure 2 shows the temperature dependences of the redox potentials for **1** and **2** in DMF and Figure 3 indicates those in dichloromethane. In DMF, the redox potentials of both complexes were unchanged on variation of temperatures. The difference in redox potentials between **1** and **2** is about 0.05 V, which is caused by the high hydrophobicity of the Ile residue in **2** around the Cys thiolate ligand in comparison with that of the Gly residue in **1**. In the case of *a dichloromethane solution*, **1** shows a temperature dependence for the redox potential. Thus,

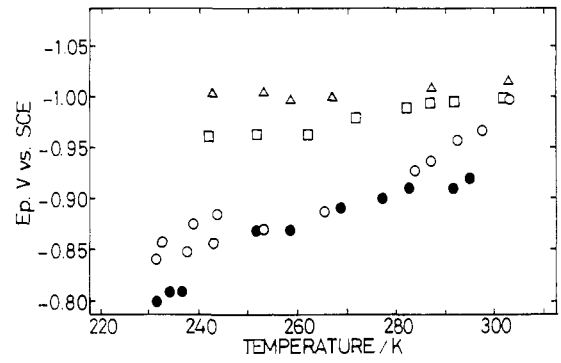


**Figure 3.** Temperature dependence of redox potentials of $[Fe_4S_4(Z\text{-}Cys\text{-}X\text{-}Ala\text{-}Cys\text{-}OMe)_2]^{2-}$ (X = Gly, Ile) in dichloromethane: (●) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]$; (□) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Ile\text{-}Ala\text{-}Cys\text{-}OMe)_2]$; (△) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}OMe)_4]$; (○) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}OMe)_4]$.

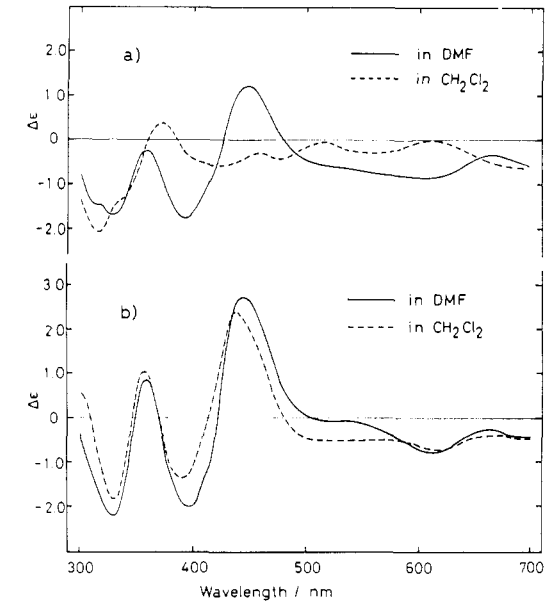


**Figure 4.** CD spectra of (a) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]$ in DMF or in dichloromethane and (b) $[NEt_4]_2[Fe_4S_4(Z\text{-}Cys\text{-}Gly\text{-}Ala\text{-}Cys\text{-}OMe)_2]$ in DMF or dichloromethane.

the redox potentials of both complexes in dichloromethane were shifted to the positive side, about 0.12 V for **1** and only 0.03 V for **2** when the temperature was lowered to −40 °C. The slopes of the redox potentials against temperature for **1** and **2** in dichloromethane are 1.7 and 0.4 mV/K, respectively.

**CD Spectra of 1 and 2.** The CD spectrum of **1** in DMF remarkably differed from that in dichloromethane, while the CD profile of **2** in DMF was similar to that in dichloromethane, as shown in Figure 4. The observed results should be ascribed to a steric restriction by the Ile residue in the macroring chelation. However, the Gly residue in **1** causes a considerable loosening of the chelate ring. Therefore, **1** is more susceptible to a solvent effect than is **2**. The solvent dependence of CD spectra in **1** is due to a conformational change arising from an interaction between the peptide and the solvent.

## Discussion

**Chelation of Cys-X-Y-Cys to $Fe_4S_4^{2+}$.** The chelation of tetrapeptides, Cys-X-Y-Cys, to the [4Fe-4S] cluster was established by the observation of two sets of the characteristic $^1H$ NMR signals of Cys $C_\beta H_2$. The [4Fe-4S] complexes of tripeptides or dipepetides containing one Cys residue exhibit two peaks due to Cys $C_\beta H_2$ at around 12–14 ppm in $Me_2SO\text{-}d_6$ or dichloromethane-$d_2$. The proton signals of Cys $C_\beta H_2$ were reported to be observed at 12.3 and 13.6 ppm in a simple peptide model complex, $[Fe_4S_4(Ac\text{-}Cys\text{-}NHCH_3)_4]^{2-}$, by Que et al.[19] These

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signals can be assigned to an ABX type of $C_\beta H_A H_B$ freely rotating around the $C_\alpha$–$C_\beta$ axis. The two peaks are due to nonequivalent $C_\beta H_2$ protons, and each proton is contact-shifted by the $Fe_4S_4^{2+}$ core through C–S–Fe bond.[25,27] The large separation of the nonequivalent peaks is faciliated by the angular-dependent pseudo contact shift by unpaired spin delocalized on sulfur $p\pi$ orbitals. The difference (0.7 ppm) of chemical shifts of the two peaks depends on the conformational probability of the restricted rotation around the Fe–S–C axis. Actually, $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ [28] and $[Fe_4S_4(S\text{-}m\text{-}xyl)_2]^{2-}$, [29] which have no asymmetric carbon adjacent to the S–$CH_2$ group, were reported to show a single broad peak at 13.8 ppm in $CD_3CN$ and 16.5 ppm in $Me_2SO$-$d_6$, respectively, at room temperature.

The chelation of the two Cys-containing tetrapeptides to a [4Fe-4S] cluster in $[Fe_4S_4(Z\text{-Cys-Gly-Ala-Cys-OMe})_2]^{2-}$ results in formation of two kinds of Fe–S(cys) bonds (see Figure 1). Four $^1H$ NMR peaks assignable to two sets of Cys $C_\beta H_A H_B$ should be observable in $Me_2SO$-$d_6$. A single peak at 11.7 ppm is assignable to one of them. Another three peaks were found at 12.5–14.5 ppm which are similar in the chemical shift values to those of the freely rotated $C_\beta H_A H_B$ of the [4Fe-4S] complexes of di- and tripeptides containing one Cys residue, although the average (13.5 ppm) of the chemical shifts of the two peaks is different from the normal one (ca. 12 ppm). The observation of four separate signals for two distinguishable sets of $C_\beta H_2$ in $CD_2Cl_2$ is ascribed to the difference in the contact shifts of $C_\beta H_2$ signals between the two Cys residues. Unfortunately, the assignment of all $C_\beta H_2$ signals could not be done. The difference ($\Delta\delta$ = 1.5 ppm) in the chemical shifts between the averages of the two sets is ascribed to the orientation of cysteine ligands, which is realized by the chelation of the tetrapeptides.

The solvent dependence of the $^1H$ NMR spectra of Cys $C_\beta H_2$ and the CD spectra of $[Fe_4S_4(Z\text{-Cys-Gly-Ala-Cys-OMe})_2]^{2-}$ can be interpreted to mean a flexible chelation of the peptide. The CD results support the chelating coordination of the two Cys-containing peptides to a single cluster, because the large extrema ($\Delta\epsilon$ = 1.5–2.5) at 450 nm in DMF are 2 times that of $[Fe_4S_4(Z\text{-Cys-Gly-Ala-OMe})_4]^{2-}$. When the complex has a polymeric structure, small extrema are expected. Moreover, $[Fe_4S_4(Z\text{-Cys-Ile-Ala-Cys-OMe})_2]^{2-}$ exhibited no solvent dependence of the CD and $^1H$ NMR spectra. The different behavior is ascribed to steric hindrance of the Ile residue in the macroring chelation, which is different from a loose chelation with the peptide containing a Gly residue. Such a conformational rigidity of the chelation in $[Fe_4S_4(Z\text{-Cys-Ile-Ala-Cys-OMe})_2]^{2-}$ is supported by the smaller temperature-dependent shift of the redox potential in comparison with the large shift of that of $[Fe_4S_4(Z\text{-Cys-Gly-Ala-Cys-OMe})_2]^{2-}$.

Que et al.[19] and Burt et al.[20] independently reported the $^1H$ NMR spectra of $Fe_4S_4^{2+}$ complexes of oligopeptides having the Cys-Gly-Gly-Cys sequence, which has no conformational restriction for chelation. When the Cys-Gly-Gly-Cys fragment chelates to $Fe_4S_4^{2+}$, no specific conformation is preferred because of the loose chelation. A tetrapeptide, Z-Cys-X-Y-Cys-OMe, can chelate to Fe(II)[15] or Pd(II)[14] if the interposed X and Y residues with alkyl side chains are suitable for the turn conformation. For example, Z-Cys-Ala-Ala-Cys-OMe in $[PdCl_2(Z\text{-Cys-Ala-Ala-Cys-OMe})]^{2-}$ chelates to a square-planar Pd(II) ion, but no chelation was observed with Z-Cys-Val-Val-Cys-OMe. Also Z-Cys-Pro-Leu-Cys-OMe is a preferable sequence for chelation to the Fe(II) ion. Thus, the X-Y fragment interposed between two Cys residues is known to play a crucial role for chelation. Therefore, the invariant sequence of Cys-X-Y-Cys in many metalloproteins controls the properties of the iron–sulfur cluster by fostering either loose or rigid chelation.

**Redox Potential Controlled by Formation of an NH---S Hydrogen Bond.** [4Fe-4S] model complexes with alkyl thiolate ligands exhibited solvent dependence of the LMCT absorption maxima in the range 390–420 nm. Generally, the LMCT absorption of the simple $Fe_4S_4^{2+}$ model complexes is blue-shifted with an increase in the dielectric constant of solvent because of solvation to the $[Fe_4S_4(SR)_4]^{2-}$ core. The blue shift of the LMCT maxima and the positive shift of the redox potentials of **1** and **2** in dichloromethane are ascribed to the NH---S hydrogen bond of Ala NH to Cys sulfur. An NH---S hydrogen bond is faciliated by solvent having a low dielectric constant such as dichloromethane. In addition to the conformational restriction by chelation of the Cys-X-Y-Cys sequence, the formation of an NH---S hydrogen bond contributes to the frozen conformation of the Cys residue, which gives four separate $^1H$ NMR signals due to $C_\beta H_2$ (Figure 1). Conformational freezing into a conformer containing NH···S hydrogen bonds is also supported by the CD spectral difference between DMF and dichloromethane (Figure 4). The previous work on $[Fe_4S_4(Z\text{-Cys-Gly-Ala-OMe})_2]^{2-}$ indicated that the linear tripeptide ligand can form the NH---S hydrogen bond with a preferable hairpin turn in dichloromethane.[8] The chelating Cys-Gly-Ala-Cys peptide in **1** has the same fragment of Cys-Gly-Ala as in $[Fe_4S_4(Z\text{-Cys-Gly-Ala-OMe})_2]^{2-}$. The chelation forces the formation of the preferable conformation for NH---S hydrogen bonding (Ala NH with Cys sulfur), which is also supported by a CPK model. On the other hand, DMF breaks the NH---S hydrogen bond with its strong solvation to the peptide amide group in the case of **1**. Thus, no temperature dependence of the redox potentials in **1** was observed. Johnson et al. reported that $[Fe_4S_4(SC_6H_4\text{-}o\text{-}OH)_4]^{2-}$ exhibits a blue-shifted LMCT absorption maximum and a positive-shifted redox potential even in DMF with the formation of OH---S hydrogen bond.[31] Such a hydrogen bond formation in DMF is probably due to the existence of OH in the vicinity of SR, different from the specially orientated NH---S hydrogen bond supported with a preferable conformation in **1**.

In the case of **2**, the Cys-Ile-Ala-Cys peptide is not suited to the formation of an NH---S hydrogen bond between Ala NH and Cys sulfur. A $\beta$-II-like hairpin turn conformation of the peptide is necessary for the NH---S hydrogen bond formation.[8,32] However, the Ile residue prevents the NH---S hydrogen bond formation, and only a weak NH---S hydrogen bond exists in **2** since the blue shift of LMCT absorption maximum of **2** occurs in dichloromethane. The chelating conformation with the steric hindrance of the Ile residue is unfavorable for the formation of the NH---S hydrogen bond. This is supported by the unchanged redox potentials with decrease in temperature. The more negative redox potential of **2** in DMF or in dichloromethane in comparison with that of **1** is ascribed to the low dielectric constant of the hydrophobic environment produced by the bulky side chain of the Ile residue. The formation of the NH---S hydrogen bond of **1** in dichloromethane leads to the positive shift of redox potentials. The steep slope (1.7 mV/K) of the temperature-dependent redox potential indicates that the peptide ligand of **1** assumes a conformation containing a NH---S hydrogen bond at low temperature.

Two effects of the NH---S hydrogen bond are thought to be responsible for the positive shift of the redox potentials. First is the direct effect of lowering the electron density at the sulfur lone pair by the NH---S hydrogen bond. This results in weakening of the Fe–S bond. Such an effect has been proposed by Sheridan et al. for native ferredoxins.[33] Another is a control of Fe–S bond characters, which is induced by the preferred orientation of Fe–S–C restricted by the chelation of two Cys-containing peptide.

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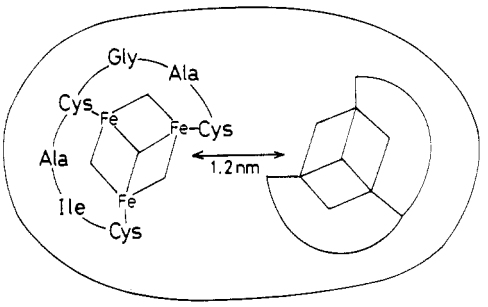


**Figure 5.** Chelation of Cys-Ile-Ala-Cys-Gly-Ala-Cys sequence to the $Fe_4S_4^{2+}$ cluster in *P. aerogenes* ferredoxin.[32]

The orientation is facilitated by the formation of the NH---S hydrogen bond and probably influences the strength of the Fe–S bond with the variation of S*–Fe–S–C torsion angles.

**Role of Invariant Sequence around $Fe_4S_4^{2+}$ in Bacterial Ferredoxins.** *P. aerogenes* ferredoxin has a characteristic sequence, Cys-Ile-Ala-Cys-Gly-Ala-Cys, which chelates in tridentate fashion to $Fe_4S_4^{2+}$. The first half of the sequence causes a negative shift of redox potential, but the later half tends to shift it positively. The remarkable difference of the redox potentials between **1** and **2** in dichloromethane suggests that a cluster of the native [4Fe-4S] ferredoxin has Fe ions with different environments, since a part of $Fe_4S_4^{2+}$ core is surrounded by Cys-Gly-Ala and the other by Cys-Ile-Ala sequences. The extremely positive shift of redox potential of **1** suggests that the peptide conformation of **1** in dichloromethane at low temperature (−40 °C) corresponds to a folding conformation of the later half (Cys-Gly-Ala-Cys), with NH---S hydrogen bonds that are supported by the hydrophobic side chains surrounding the peptide chain. Another hydrophobic peptide complex, $[Fe_4S_4(Z\text{-Cys-Pro-Val-OMe})_4]^{2-}$ having the sequence of the $Fe_4S_4^{2+}$ core of *P. aerogenes* ferredoxin, exhibits a relatively negative redox potential (−1.34 V vs. SCE in DMF).[34] An X-ray analysis of *P. aerogenes* ferredoxin by Adman et al.[32] indicates that the Cys-Ile-Ala-Cys sequence is located far from another $Fe_4S_4^{2+}$ core (cluster II) and the Cys-Gly-Ala-Cys sequence is between the two $Fe_4S_4$ cores while the Cys-Pro-Val sequence is on one side of cluster I as shown in Figure 5. Such an inequivalence within the $Fe_4S_4^{2+}$ core may play an important role in electron transfer, especially in influencing the direction of flow of electrons in biological electron-transfer chains.

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# Potentiometric Study of the Solvent Equilibria in $AlCl_3$–*N*-*n*-Butylpyridinium Chloride Melts

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The solvent acid–base properties of acidic ($AlCl_3$-rich) $AlCl_3$–*N*-*n*-butylpyridinium chloride melts have been investigated by potentiometry for compositions up to 2.15:1 molar ratio $AlCl_3$:BuPyCl (the upper composition limit depends on the temperature). The ionic species distribution in this molten salt system is fully described by the following two equilibrium reactions: (I) $2AlCl_4^- \rightleftharpoons Al_2Cl_7^- + Cl^-$; (II) $2Al_2Cl_7^- \rightleftharpoons Al_3Cl_{10}^- + AlCl_4^-$. Equilibrium I is shifted completely to the right (i.e. formation of $Al_2Cl_7^-$) in acidic melts. The value of the equilibrium constant $K_{II}$ was determined as 2.09 (±0.06) × $10^{-3}$ (40 °C), 2.93 (±0.02) × $10^{-3}$ (60 °C), and 4.01 (±0.07) × $10^{-3}$ (80 °C); $\Delta H^\circ$ = 15.20 (±0.28) kJ and $\Delta S^\circ$ = −2.86 (±0.86) J/deg.

## Introduction

Mixtures of aluminum chloride and *N*-*n*-butylpyridinium chloride (BuPyCl) are ionic liquids at ambient temperatures (the system is liquid below 27 °C over the composition range 0.75:1 to 2:1 molar ratio of $AlCl_3$:BuPyCl).[1,2]

Gale and Osteryoung[3] and, later, Schoebrechts and Gilbert[4] concluded from potentiometric measurements on concentration cells of the type

$$Al|BuPyCl,AlCl_3(ref):fritted\ disk:AlCl_3,BuPyCl|Al \quad (1)$$

that the Lewis acid–base properties of this low-temperature molten salt can be represented by the single equilibrium reaction

$$2AlCl_4^- \rightleftharpoons Al_2Cl_7^- + Cl^- \quad (I)$$

It is difficult to obtain accurate values of $K_I$ from potentiometric measurements on concentration cells of type 1 because of the oxidation of aluminum metal by the organic cation in basic (chloride-rich) melts. The value of $K_I$ deduced by Karpinski and Osteryoung[5] from voltammetric measurements, log $K_I$ = −16.9 (±2) at 40 °C, seems to be the more acceptable estimate (this value is several orders of magnitude smaller than the values determined by potentiometry[3,4]).

The conclusion that the single equilibrium reaction I provides an adequate description of the system throughout the entire range of melt compositions up to a 2:1 molar ratio of $AlCl_3$:BuPyCl seems to be supported by $^{27}Al$ NMR[6,7] and Raman[8] spectral results (none of the more intense Raman bands of liquid or gaseous aluminum chloride could be ascertained in the spectra, even in acidic ($AlCl_3$-rich) melts; this is consistent with the observation that sublimation losses of aluminum chloride are minimal even at high temperatures). Nevertheless, there are several reasons why it is necessary to consider additional equilibria, involving species such as $AlCl_3$, $Al_2Cl_6$, $Al_3Cl_{10}^-$ (which all are known to exist in inorganic chloroaluminates), for the most acidic melt compositions. Thus, systematic deviations are observed between the experimental and theoretical potentials, increasing with the melt acidity for compositions higher than ~1.8:1 molar ratio of $AlCl_3$:BuPyCl, if the theoretical potentials are calculated on the

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